OPP-2002-0293

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Additional Data on Woad Warrior (*Puccinia thlaspeos* 'woad strain' on *Isatis tinctoria*)

TO: Barbara Mandula
Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

FROM: John L. Kough, Senior Scientist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

5/17/02
(signed)

ACTION REQUESTED: To review the additional data on host range and manufacturing process to justify a registration for Woad Warrior.

CONCLUSION: The data submitted continue to support the supposition that Woad Warrior specifically infects and kills dyer's woad. This report clarifies the manufacturing process including product quality control and includes an updated list of the species of members in the Brassicaceae that have been tested for susceptibility to the fungus in Woad Warrior.

DATA REVIEW RECORD

Active Ingredient:	Dyer's woad rust (*Puccinia thlaspeos* 'woad strain' on *Isatis tinctoria*)
Product Name:	Woad Warrior
Company Name:	Greenville Farms, North Logan, Utah
ID No:	73417-R
Chemical Number:	6489
Submission Number:	S608118
DP Barcode:	D280230
MRID No:	455509-01- Product Characterization

455509-02- Non-target Organism Safety

BACKGROUND: Greenville Farms has had an EUP to examine the efficacy of Woad Warrior as a mycoherbicide on dyer's woad. The product would be used predominately in the Intermountain region where dyer's woad is a pernicious weed especially in areas where chemical herbicides would be too costly or damaging. The apparently abrupt appearance of this rust fungus on dyer's woad and the inability to account for its origins have been the primary concern for this biopesticide.

DISCUSSION: The submitted data has addressed the major issues mentioned in the original review for the EUP (memorandum from J. Kough to B. Mandula, Feb 20, 2001). Still outstanding is the request to more thoroughly address the phylogeny of the dyer's woad rust in relation to the *Puccinia monoica* group of fungal species and include a report on the examination of native crucifers **and grasses** for rust infections during the EUP.

RECOMMENDATION: The data supports a finding of no unreasonable adverse effect to man or the environment for use of this fungus to control dyer's woad. It is recommended that the company continue to closely monitor surrounding vegetation for signs of rust infection and report to EPA any instances of infected plants other than dyer's woad that appear to be infected with the *Puccinia thlaspeos* 'woad strain' fungus.

SUMMARY OF DATA SUBMITTED:

455509-01: The report briefly identifies the only ingredient of Woad Warrior as leaf pieces of *Isatis tinctoria* infected with the rust, *Puccinia thlaspeos* 'woad strain'. The active ingredient is the teliospores of *P. thlaspeos*. The manufacture of Woad Warrior is from field-grown dyer's woad infected with the rust. This process includes a method by which samples will be analyzed for quality assurance, and the definition of upper and lower limits. An explanation is provided as to why no viable unintentional plant contaminants are expected to be present in the product.

455509-02: The volume is divided into five parts. The second and major part of the study addresses potential toxicity to nontarget plant species. The results of nontarget plants studies are presented in summary form. Variances with OPPTS 885.4300 include a lack of study details and limitation of testing to plant species in the Brassicaceae. The latter appears justified, based on available data indicating that dyer's woad rust specifically infects *Isatis tinctoria* (dyer's woad) and would present no danger to other plants in the vicinity when used to control dyer's woad. The study also presents evidence from the scientific literature and/or personal observations for the lack of potential toxicity of dyer's woad rust to humans and animal species. Indirect effects of rust infection on some herbivorous insects feeding on the rusted plants has been reported in the literature. These cited effects (e.g., feeding , oviposition, longevity, egg production, egg hatch and larval development) could probably be related to the decreased vigor of the host plant providing a less desirable food base rather than a direct effect of the rust fungus itself. It is also important to note that the citations are for other rust species (*Puccinia carduorum* and *Puccinia lagenophorae*).

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Primary Reviewer: Isabel Mandelbaum, Ph.D, DABT Signed: March 11, 2002
Tetrahedron Contractor

EPA Secondary Reviewer: John Kough Date 5/17/02

Data Evaluation Record

STUDY TYPE: Product Identity and Manufacturing (151A-10,-11,-12,-13,-15)

CHEM. NO.: 006489

DP BARCODE: D280230

CASE No.: 071715

P.C. CODE: 073417-R

SUBMISSION CODE: S608118

TEST MATERIAL: *Puccinia thlaspeos* 'woad strain' teleosori on *Isatis tinctoria* (dyer's woad)

SYNONYMS: Woad Warrior

MRID No.: 455509-01

CITATION: Thomson, S. (2001). Volume 2, Product Identity. Greenville Farms (N. Logan, Utah)). No report number, November 13, 2001. MRID 45550901. Unpublished.

SPONSOR: Greenville Farms
1689 N. 1200 E.
N. Logan, Utah 84341

SUMMARY: The report briefly identifies the only ingredient of Woad Warrior as leaf pieces of *Isatis tinctoria* infected with the rust, *Puccinia thlaspeos* 'woad strain'. The active ingredient is the teliospores of *P. thlaspeos*. The manufacture of Woad Warrior from field-grown rust, including the method by which samples will be analyzed for quality assurance, and the definition of upper and lower limits is provided. An explanation is provided as to why no viable unintentional plant contaminants are expected to be present in the product.

COMPLIANCE: A dated, unsigned statement is included that indicates that the study did not comply with Good Laboratory Practices (GLPs) because records were not kept in accordance

with GLP guidelines. A dated, unsigned statement of no data confidentiality claims is provided.

The report was prepared in accordance with OPP guidelines 151A-10, -11, -12, -13, -15. These guidelines have been harmonized into OPPTS 885.1100 through 1500.

A. PRODUCT IDENTITY

The product is identified as composed of teleosori of the rust *Puccinia thlaspeos* 'woad strain' on leaf pieces of *Isatis tinctoria* (dyer's woad). The product size is 1-2 mm pieces of leaves and stems of *I. tinctoria*. The active ingredient is the teliospores that are present in the teliosori; the leaves and stems are inert. Basidiospores, which are produced from germinated teleospores are short-lived and reported not to be present in large numbers in the product.

B. MANUFACTURING PROCESS

Woad Warrior is prepared from field-grown, naturally infected dyer's woad plants. The plants are harvested by breaking the stems to avoid contamination with root material. The plants are dried at 32°C and ground using a Wiley mill into pieces approximately 1 to 2 mm in size.

Each batch will be analyzed to determine the number of teliosori on five 0.01 g samples of 1-2 mm leaf pieces. Previous studies have shown an average of 4.32 teliosori per leaf piece; the average number of leaf pieces with teliosori is 74.4 out of 90 in 0.01 g which is 80% of the dry weight of Woad Warrior. Each teliosorus has an average of 645 teliospores, resulting in an average 20,619,360 teliospores per gram or 9.4×10^9 teliospores per pound of Woad Warrior.

The teliospores will be checked for viability by placing leaf pieces on a plate of water agar and then inverting the plate over an agar block to capture the basidiospores. The presence of basidiospores on the block indicates the teliospores are viable and of good quality.

Woad Warrior is stored in plastic bags at 5°C until use; teliospores can be stored at 5°C for up to 6 months without significant loss of viability; the quality may decline about 50% after one year of storage, but viable teliospores capable of causing infection are still produced.

C. FORMATION OF UNINTENTIONAL INGREDIENTS

Plants used in the preparation of Woad Warrior are chlorotic and have cinnamon colored pustules on the leaves; no other plant conditions or diseases could be confused with plants infected with woad rust.

A powdery mildew (tentatively identified as *Erysiphe sp.* has been seen on plants grown in greenhouse studies; however, it is not commonly found on field-grown dyer's woad plants. It is

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most often observed on rosettes and not on bolting plants where teliospores are produced; therefore, it is unlikely that conidia of the mildew would be present when the rusted plants were harvested. The mildew conidia are short-lived (resistant cleistothecia are not produced), and the manufacturing process (drying, grinding, and storage at 5°C) would eliminate viable conidia.

Based on the scientific literature and the author's personal observations, no other pathogenic plant fungi are known to be present on dyer's woad within the U.S. *Isatis tinctoria* is not listed in the Host index "Fungi On Plants and Plant Products in the United States (Farr et al., 1989). The cabbage aphid, *Brevicoryne brassicae*, is occasionally present on a few plants; any living insects would be eliminated by the drying and grinding process.

D. ANALYSIS OF SAMPLES

Each batch of Wood Warrior will be analyzed to determine the number of leaf pieces with teliosori on five 0.01 g of 1-2 mm size leaf pieces. Previous studies have shown an average of 4.32 teleosori per leaf piece, and an average of 645 teliospores per teliosori. Based on this information, the number of teliosori per gram of ground leaf tissue will be estimated.

Teliospores will be checked for viability by placing leaf pieces on a plate of water agar and inverting the plate over an agar block to capture the basidiospores. The viability and good quality of the teliospores will be indicated by the presence of basidiospores on the block .
Staining of the agar block with acid fuchsin may be used to aid in counting the basidiospores.

E. CERTIFICATION OF LIMITS

Ground pieces of rust-infected *I. tinctoria* are the only ingredient in the Woad Warrior formulation.

The lower limit is to be set at 60 leaf pieces with teliosori per 0.01 g; it is stated that this would yield approximately 16,848,00 teliospores/gram or 7.6×10^9 teliospores per pound of Woad Warrior (note: based on the previously stated averages of 4.32 teliosori/leaf and 645 teliospores per teliosori, a value of 16,718,400 would be calculated).

The lower and upper limits of leaf pieces with teleosori, are 72% and 88%, respectively, based on the means of over 10 batches of Woad Warrior. This range of limits also indicates the range of weight of leaf pieces with teliosori, because leaf pieces are consistent in size. The leaf pieces are reported to make up approximately 80% by weight of Woad Warrior.

Each teliospore germinates to produce 4 basidiospores. Usually 95% of the teliosori produce basidiospores within 24 hours and production continues but declines after 72 hours. The application rate of Woad Warrior ranges from 7.6 to 16.8 kg/hectare (7 to 15 pounds/acre).

C. DISCUSSION

The report briefly identifies the product. More specific information regarding the identity of *P. thlaspeos* 'woad strain' is presented in Volume 3 of the report (MRID 455509-02).

Although an explanation of why no unintentional viable plant contaminants are likely to be present is presented , no specific information is provided describing the general environment in which the product will be manufactured, and how general contamination of the product may be avoided during its manufacture.

Primary Reviewer: Isabel Mandelbaum, Ph.D, DABT Signed: March 11, 2002
Tetrahedron Contractor

EPA Secondary Reviewer: John L. Kough, Ph.D., Date 5/17/02

Data Evaluation Record

STUDY TYPE: Nontarget Plant Studies, Tier 1 (OPPTS 885.4300)

CHEM. NO.: 006489

DP BARCODE: D280230

CASE No.: 071715

ID NUMBER: 073417-R

SUBMISSION CODE: S608118

TEST MATERIAL: *Puccinia thlaspeos* 'woad strain' teleosori on *Isatis tinctoria* (dyer's woad)

SYNONYMS: Woad Warrior

MRID No.: 455509-02

CITATION: Thomson, S. (2001). Volume 3, Plant Toxicity Data Requirements. Greenville Farms (N. Logan, Utah). No report number, November 13, 2001. MRID 455509-02. Unpublished.

SPONSOR: Greenville Farms
1689 N. 1200 E.
N. Logan, Utah 84341

SUMMARY: The volume is divided into five parts. The second and major part of the study addresses potential toxicity to nontarget plant species. The results of nontarget plants studies are presented in summary form. Variances with OPPTS 885.4300 include a lack of study details and limitation of testing to plant species in the Brassicaceae. The latter appears justified, based on available data indicating that dyer's woad rust specifically infects *Isatis tinctoria* (dyer's woad) and would present no danger to other plants in the vicinity when used to control dyer's woad. The study also presents evidence from the scientific literature and/or personal observations for

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the lack of potential toxicity of dyer's woad rust to humans and animal species. Indirect effects of rust infection on some herbivorous insects feeding on the rusted plants has been reported in the literature. These cited effects (e.g., feeding , oviposition, longevity, egg production, egg hatch and larval development) could probably be related to the decreased vigor of the host plant providing a less desirable food base rather than a direct effect of the rust fungus itself. It is also important to note that the citations are for other rust species (*Puccinia carduorum* and *Puccinia lagenophorae*).

COMPLIANCE: A statement is included that indicates that the study did not comply with Good Laboratory Practices (GLPs) because records were not kept in accordance with GLP guidelines. A statement of no data confidentiality claims is also provided.

A. STUDY DESIGN

The volume was divided into five main sections, titled as follows:

1. Safety and Toxicity Concerns in the Preparation of Inoculum

This section included the following subsections:

Toxicity to Workers

No adverse health effects, including skin and eye irritation, have been reported on workers involved in the manufacturing or application processes. This lack of toxicity to workers was based on observations during the preparation (gathering, drying), mixing (grinding), and application (spraying, dusting) of the product. A literature reference about the etiology of dermatitis during wheat harvest is cited that indicates the *Puccinia* species present in these samples was shown not be the cause of the reported dermatitis.

Effects on Other Organisms

Potential toxicity of Woad Warrior to animal species was addressed by a literature search and by personal observations. Five databases were searched: Agricola; Current Contents; Biosis; Biological and Agricultural Abstracts; and Applied Science and Technology Abstracts. The databases were searched for information regarding *Puccinia* and animals, in general. Specific searches were made for reports that included *Puccinia* and any of the following: animals, birds, mammals, fish, and insects.

According to the author, the literature search did not reveal any adverse effects of *Puccinia sp.* on birds, mammals, or fish; because no animals studies were cited, it is assumed that no animal toxicity studies were located. The author reported that in field studies, deer have occasionally been observed to feed selectively on infected dyer's woad. In a preliminary study on captive deer, the deer showed no obvious preference, and those

that did consume infected plants exhibited no ill effects. Mice observed in the greenhouse were also reported to show a preference for infected plants. Few herbivores have been observed to eat woad; sheep and deer occasionally eat flowers, but cattle and other grazing animals avoid it.

Two studies were cited from the literature indicating effects of *Puccinia* sp. (but not dyer's woad rust) on some herbivorous insects, including effects on feeding, oviposition, longevity, egg production, egg hatch, and larval development. It is important to note that these effects were not lethal but rather delayed development.

Historical Authenticity of Woad Rust Collections

A collection of woad rust teliospores is being maintained for future comparisons with natural field populations. Specimens of infected *Isatis tinctoria* have been deposited in the Arthur Herbarium, Purdue University (Nos. PU N2489, PU N2490) and the Intermountain Herbarium at Utah State University (Nos. 143904, 143906).

DNA sequences of the ITR region have been determined. The collections can be used for future morphological or DNA comparisons.

Insects or Diseases of Woad

Powdery mildew (*Erysiphe* sp.) has been observed growing on woad in the greenhouse, but rarely in natural stands. Cabbage aphids have been observed on foliage during some years.

Conditions Necessary for Woad Rust Infection

In general, studies indicate that natural infection occurs during April to July when the plants are still in the rosette stage of development. Symptoms may be expressed in as little as 9 weeks, but usually take 1 year. Fall infection can be produced artificially but is rare in nature. The target is dyer's woad rosettes in the first year of growth. Applications to more mature or plants tha have bolted will not result in infection. While infected plants can be killed outright, the more typical response from the initial infection is stunting and decreased seed production.

When intact teliosori are suspended over water agar, basidiospores are readily produced between 3 and 6 hours at 10 to 20°C with adequate moisture. The optimum temperature for basidiospore production is 15°C with spores being produced as low as 5°C, but not at 25°C. In the field, woad rust infects in the spring when temperatures are cool and rainfall is more frequent.

Under ideal conditions in a dew chamber (i.e., 24 hours of leaf wetness at 15°C in the

dark), inoculation resulted in 94% infection. These conditions would be most similar to normal spring weather in Utah.

2. Host Range or Toxicity of *Puccinia thlaspeos* 'Woad Strain' to Non-Target Plants

Selection of Test Plant Species

The author states that the host range of the woad rust cannot be confirmed in the literature because it is a newly described rust with uncertain affinities to other related rusts. *Puccinia thlaspeos* belongs to the *Puccinia monoica* complex of rusts. It has been concluded by other investigators that the *P. monoica* complex of rust fungi contains cryptic species (i.e., there may be additional species in the group that are not yet recognizable by standard taxonomic or other criteria). The rust on *Isatis tinctoria* appears to be a variant of one of these cryptic species. Until further speciation is performed on this group, it has been advised that the name *Puccinia thlaspeos*, specified as woad rust, be used to refer to the dyer's woad rust fungus. The dyer's woad rust is an autoecious, microcyclic rust that can complete its entire life cycle on dyer's woad alone.

The plant hosts of the *P. thlaspeos* species in general are all non-agricultural species in the Brassicaceae. These hosts are listed in the report and are the same as those referenced in the original review for the EUP (memorandum from J. Kough to B. Mandula, Feb.20, 2001). Based on the scientific literature, most of hosts of the original *P. thlaspeos* are closely related genera in the Brassicaceae, common wild mustards that are widely distributed. Hosts for *P. consimilis* and *P. monoica*, included in the *P. monoica* complex, also are listed, because there is no clear separation of these species from *P. thlaspeos*. None of the hosts listed for either *P. thlaspeos* or *P. consimilis* are agricultural crops. *P. monoica* has several grass species listed as telial hosts.

Host Range Studies

Studies that have been performed by the author and associates to determine host susceptibility were presented in summary form. Woad rust has been tested on Brassicaceae species that have been reported to be hosts of rusts in the *P. monoica* complex.

In 1993 and 1994, 30 pots each of *Descurainia pinnata*, *Smelowskia calycina*, *Thlaspi montanum*, *Thlaspi arvense*, *Brassica napus* 'Westar", and *Brassica napus* 'Goldrush', along with 30 pots of healthy dyer's woad, were placed in a field site alongside naturally-occurring infected woad. In 1993 and 1994, 81% and 69% of the potted dyer's woad, respectively, became infected. None of the other plants tested except dyer's woad showed any symptoms of infection. *D. pinnata*, *S. calycina*, and *T. montanum* have been identified as hosts of *P. thlaspeos*. *T. arvense* was included because it often occurs in

woad-infested areas. The two *Brassica* sp. (canola) were tested because canola is a close relative of the wild mustards and is an agriculturally important crop in some regions where dyer's woad is a noxious weed.

In a greenhouse study, one month-old *B. napus* plants (15 plants each of 'Westar' and 'Goldrush') and 15 dyer's woad were inoculated in the laboratory with ground dyer's woad leaf pieces bearing fungal teleosori. Woad rust is an obligate parasite and cannot be grown on artificial media. Leaf pieces were spread on water agar plates, which were inverted over the center of the plant. The plants were placed in a dew chamber with dew formation and incubated in the dark at 15°C; the plants were moved to the greenhouse after 24 hours. Thirty-three percent of the dyer's woad plants developed symptoms of infection, while none of the canola plants showed any symptoms.

In a field study, seeds of dyer's woad and canola were sown together in six 2 ft x 2 ft plots; each plot was separated by 10 feet to prevent contamination. When mature leaves developed (approximately one month), the plants were thinned to approximately 25 canola and 25 dyer's woad per plot. All plants in 3 plots were inoculated by dusting with ground dyer's woad leaf pieces bearing fungal sori, the remaining 3 plots were uninoculated controls. Canola, an annual, was harvested after bolting in the fall; the dyer's woad was allowed to overwinter and examined in the spring. The canola did not exhibit any symptoms of infection; 10% of the dyer's woad became infected. There was no significant difference in dry weights between the canola plants grown near dyer's woad and plants exposed to the rust.

Twenty plants of *Sisymbrium altissimum*, 20 plants of *Sisymbrium austriacum*, 13 plants of *Erysimum cheiranthoides*, and 10 plants of dyer's woad were grown from seed in the greenhouse, and inoculated as described above for the greenhouse studies on canola. After 2 months in the greenhouse, 30% (3/10) of the dyer's woad plants showed symptoms; none of the other plants showed signs of infection.

In all recent tests, plants were grown from seed in the greenhouse until several leaves were present. Inoculum of the woad rust was prepared from ground, infected leaf material containing teliospores of *P. thlaspeos* 'woad strain'. Leaf pieces were placed on water agar plates and inverted over at least 15 plants. Plants were placed in a dew chamber at 15°C in the dark for 24 hours; 15 plants of dyer's woad were inoculated at the same time. Plants were then moved to the greenhouse until acclimatized and then transplanted outside to a raised bed until symptoms appeared in the woad plants. In most trials, at least 33% of the woad plants became infected within 2-6 months. No sign of infection has been observed in any other plant test species.

Representative species of all related genera in the Brassicaceae have been tested; if an exact species listed in a host index could not be found, another species in the genus was tested.

The following species of *Arabis* also have been inoculated with woad rust, but the results have not yet been determined: *A. beckwithii* Rollins, *A. cobrensis* M.E. Jones, *A. davidsonii* Greene var. *davidsonii, A. demissa* Greene var. *languida* Rollins, *A. drummondii* Gray, *A. falcifructa* Rollins, *A. fernaldiana* Rollins var. *stylosa* (S. Wats.) Rollins, *A. holboellii* Hornem. var. *retrofracta* (Graham) Rydh, *A. lasiocarpa* Rollins, *A. lemmonii* S. Wats var. *drepanoloba* (Greene) Rollins, *A. lignifera* A. Nels, *A. perennans* S. Wats, *A. puberula* Nutt. ex Torr & Gray, *A. schistacea* Rollins, *A. shockleyi* Munz, *A. williamsii* Rollins var. *williamsii*.

Among other hosts species of the *P. monoica* group, seeds could not be found by the date of the report for the following species: *Arabis crandallii*, *Arabis divaricarpa*, *Arabis exilis*, *Arabis furcata*, *Arabis gunnisoniana*, *Arabis lyallii*, *Arabis lyrata*, *Arabis microphylla*, *Arabis nuttallii*, *Arabis sparsiflora*, *Sisymbrium linifolium* (= *Schoencrambe linifolia*), *Smelowskia americana*, *Thlaspi glaucum*, *Thlaspi nutallii*, and *Thlaspi pupurescens*.

Since the last review the following additional species of Brassicaceae have been tested and shown not to be discernibly infected by the dyer's woad rust: *Arabis fecunda, A. gerardii, A. hirusta, A. holboellii, A. turrita, Draba* sp., *Erysimum crepidifolium, Hesperis matronallis, Lesquerella argyraea, L. pererata, Polyctenium fremontii,* and *Rhaphanus sativus*.

Summary of Results of Host Range Studies

The the comprehensive list of host plants that have been tested for susceptibility to dyer's woad rust are found below, along with the species in the *P. monoica* group of rusts reported to be its host, where applicable. None of these plants have been shown to be susceptible to dyer's woad rust (i.e., the results have all been negative).

PLANT SPECIES TESTED (NEGATIVE RESULTS)	**NORMAL INFECTIVE SPECIES**
Arabis breweri	*P. thlaspeos*
Arabis brownii	None given
Arabis fecunda	None given
Arabis gerardii	None given
Arabis glabra	None given
Arabis hirsuta	*P. thlaspeos*; *P. monoica*
Arabis holboellii	*P. thlaspeos*; *P. monoica*

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Arabis turrita	None given
Brassica napus 'Canola'	None given
Brassica oleraceae var. *capitata* 'cabbage'	None given
Descurainia pinnata	*P. thlaspeos*
Draba sp.	*P. thlaspeos*
Erysimum sp.	*P. thlaspeos*
Erysimum asperum	None given
Erysimum crepidifdium	None given
Erysimum inconspicuum	*P. consimilis*
Erysimum cheiranthoides	None given
Erysimum repandum	None given
Hesperis matronallis	None given
Lepidium sativum	None given
Lepideum campestrus	None given
Lesquerella argyraea	None given
Lesquerella gordonii	None given
Lesquerella fendleri	None given
Lesquerella perferata	None given
Lunaria annua	None given
Polyctenium fremontii	*P. thlaspeos*
Raphanus sativus 'radish'	None given
Sisymbrium austriacum	None given
Sisymbrium altissimum	None given
Smelowskia calycina	*P. thlaspeos*
Thlaspi arvense	None given
Thlaspi montanum (= *alpestre*)	*P. thlaspeos*

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Conclusion

Based on the above studies, there is no evidence to suggest that *P. thlaspeos* 'woad strain' will infect any likely plant species in the Brassicaceae other than dyer's woad.

3. Endangered Species

Based on a literature review, no hosts of the rusts in the *P. monoica* group are listed as threatened or endangered. All hosts of *P. thlaspeos* are Brassicaceae species with no alternative hosts.

There are other species of Brassicaceae that are threatened or endangered in the continental United States. A list of these is provided. Of the plants listed, those occurring in Colorado, California, and Utah (21 species) can be expected to have the same or potential geographical range as dyer's woad. These are: *Arabis hoffmannii*, *Arabis mcdonaldiana*, *Caulanthus californicus*, *Erysimum capitatum* var. *angustatum*, *Erysimum menziesii*, *Erysimum teretifolium*, *Eutrema penlandii*, *Lepidium barnebyanum*, *Lesquerella congesta*, *Lesquerella kingii*, *Lesquerella tumulosa*, *Physaria obcordata*, *Rorippa gambellii*, *Schoenocrambe suffrutescens*, *Schoenocrambe barnebyi*, *Schoenocrambe argillaceae*, *Sibaria filifolia*, *Streptanthus niger*, *Streptanthus albidus* var. *albidus*, *Thelypodium stenopetalum*, and *Thysanocarpus conchuliferous*.

Attempts to find field populations of endangered species of Brassicaceae in the vicinity of rusted dyer's woad have been unsuccessful. Species in the same genus to those listed as endangered have been tested when seed has been available; there has been no evidence of infection in the species tested thus far.

4. Rusts on *Isatis tinctoria* in Europe

Isatis tinctoria is a native of southeastern Russia, from which has spread throughout the eastern hemisphere. It has not become widespread in Europe or Asia, however, and is not considered a serious weed in Europe. *P. trabutii* (also known as *P. isiaceae*) has been listed as a rust on *I. tinctoria* in Europe. *P. trabutii* is not present in North America. *P. tabutii* is very different from, and could not be confused with, *I. tinctoria. P. trabutii* is a macrocyclic, heteroecious rust that produces aeciospores and urediospores on the Brassicaceae host, and teliospores on the alternate grass host. A table is provided that compares and contrasts the spore characteristics of the *P. monoica* group.

Woad plants grown from *Isatis tinctoria* seed collected in Europe were shown to be equally susceptible to woad rust when inoculated using the author's standard technique. The typical *P. thlaspeos*, although a fairly common rust on various Brassicaceae hosts in Europe, has not been reported on *I. tinctoria* in Europe.

Woad rust appears to be a unique strain of *P. thlaspeos*. Its uniqueness would explain why *P. thlaspeos* in Europe doesn't infect European *I. tinctoria*, and explain why woad rust does not infect other Brassicaceae hosts in the western United States.

5. Systematics of Puccinia on Brassicaceae Hosts

This section present a short discussion regarding the *P. monoica* complex of rust fungi and their hosts. The *P. monoica* rusts are common in the northern hemisphere and attack about 960 species of crucifers in 11 genera.

Woad rust appears to be most similar to *P. thlaspeos* because it infects a Brassicaceae host and has the same spore types and morphology as *P. thlaspeos*; however, efforts to infect any of the common host plants of *P. thlaspeos* have yielded negative results. It appears that *P. thlaspeos* is polyphyletic and probably includes multiple species (i.e., genetically different fungi that cannot be separated morphologically).

In July 2000, plants of *Arabis lasiocarpa* (= *Boechera lasiocarpa*) growing near Willard Peak, Utah were observed to be infected with a rust. This was the first time the author had observed another rust-infested brassicaceous plant in the vicinity of rusted woad. Cross-inoculation studies were carried out; dyer's woad rust did not infect the *A. lasiocarpa* plants and the rust on *A. lasiocarpa* did not infect dyer's woad. DNA sequencing of the ITS region from the rust on *Arabis* revealed that it was not the same as the ITS sequence of dyer's woad rust. It was concluded that the rust on *A. lasiocarpa* was a typical *P. thlaspeos* and not woad rust.

With the exception of the *A. lasiocarpa*, over a 10-year period, close inspection by the author of other plants growing in the vicinity of rust-infected dyer's woad has resulted in no observations of rust infection in any other plant species.

B. DISCUSSION

The studies were reportedly conducted in accordance with OPPTS 885.4300. A number of variances from the guidelines were noted.

The nontarget plant studies were presented in summary form and a number of study details were absent. For example, descriptions of the ambient conditions in the greenhouse or in the field following inoculation were not provided. The summaries for most of the studies did not indicate that a negative control (i.e., uninoculated dyer's woad) was included. No discussion was provided of the dose levels used in the studies, or how those dose levels compared with those that would be used for commercial application. No description was provided of the criteria that were used to determine infected plants from those that were uninfected. The frequency of observations was not

reported and the study duration was not reported in all cases. Exact start and termination dates of the studies were not included. No details were provided of the history of the test plants/seeds from which the test plants were grown.

Testing was limited to members of the Brassicaceae. Although OPPTS 885.4300 indicates more diverse testing should be performed, the scientific literature and long-term observations have provided no reason to believe that other plant families have any reasonable likelihood of serving as hosts to woad rust.